A vascular view on cognitive decline and dementia

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Chapter 2.1

Specific risk factors for microbleeds and white matter hyperintensities in Alzheimer's disease

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Abstract

We investigated whether microbleeds and white matter hyperintensities (WMH) in Alzheimer's disease (AD) associate more with conventional vascular risk factors or with risk factors that reflect amyloid burden. We included 371 patients with probable AD. WMH (Fazekas 2 or 3) were present in 107 (29%) patients and microbleeds were seen in 98 (26%). Patients with both microbleeds and WMH appeared to be older and presented more frequently with lacunes and multiple microbleeds than patients with microbleeds in isolation (all $p<0.05$). Using multivariate regression models, we found that WMH presence showed independent associations with age, hypertension, current smoking, and lacune presence. Microbleeds were independently associated with male gender, higher blood pressure, lower CSF Aβ42, and ApoE ε4 homozygocity. Separate analyses for microbleeds according to their location showed that these associations were driven by microbleeds in lobar locations. Our results suggest that, unlike WMH, microbleeds in AD are particularly associated with additional amyloid burden, and as such, may relate to cerebral amyloid angiopathy (CAA).

Introduction

Alzheimer's disease (AD) is essentially regarded as a neurodegenerative disorder, but increasing evidence suggests that vascular disease may play a role as well.[1-6] Cerebral amyloid angiopathy (CAA) and small vessel disease related to conventional vascular risk factors may both explain vascular disease in AD.[7]

Microbleeds and white matter hyperintensities (WMH), both regarded as MRI manifestations of vascular disease, are more frequently observed in AD patients compared to a general elderly population.[8,9] Microbleeds are presumed to reflect focal haemosiderin deposits resulting from small vessel blood leakage[10] and they have been associated with CAA as well as small vessel disease.[11,12] WMH are assumed to be rather heterogeneous in nature and severity[13] and may also be seen in CAA.[14,15] In elderly populations, however, WMH are generally considered a consequence of small vessel disease, and are particularly associated with hypertension.[16]

On MRI, microbleeds may occur with or without additional WMH. Specifically microbleeds in non-lobar locations have been associated with WMH and these microbleeds are presumed to reflect small vessel disease.[11,12,17] Previously, we observed that in AD patients ApoE ε4 carriers with multiple microbleeds presented with remarkably little WMH.[18] Research in a community-based setting linked ApoE ε4 to microbleeds in lobar locations and suggested CAA as underlying substrate.[11,12]

At autopsy CAA is found in 80%-95% of the AD cases.[19] Most of these patients only have a mild form; approximately 25% have severe CAA.[20] Presence of CAA has important implications for anti-amyloid immunization trials. Both the presence of CAA at study entry, and a transient increase in CAA by immunization, may predispose to adverse treatment responses, called amyloid related imaging abnormalities (ARIA).[21-23] At present it is therefore recommended that microbleeds are carefully detected at baseline and during these trials, as it is assumed that microbleeds may be a sign of CAA. However, many aspects of the aetiology and clinical relevance of ARIA are still unknown and there is only limited data about the risk profiles of AD patients presenting spontaneously with microbleeds.

Our aim was to investigate whether microbleeds and WMH on MRI, in isolation or combined, associate with certain risk factor profiles in patients with AD. We were particularly interested in risk factors that reflect amyloid burden (low CSF Aβ42 and ApoE ε4 carriership) in relation to microbleeds. Firstly, we compared AD patients that presented with microbleeds in isolation to AD patients that presented with microbleeds and WMH in combination. Moreover, we investigated independent risk factors for

microbleeds and WMH. We also investigated microbleed location, as different locations may reflect different risk factor profiles.

Methods

Patients

We included patients from the memory clinic based Amsterdam Dementia Cohort. Patients visited either the Alzheimer center or the internal/geriatric medicine department of the VU university medical center Amsterdam (VUmc) between August 2008 and August 2011. All patients underwent an extensive dementia screening, including medical history, neurological and physical examination, cognitive assessment, and brain MRI. The diagnosis 'probable Alzheimer's Disease (AD)' was made according to the NINCDS-ADRDA criteria, by consensus of a multidisciplinary team.[24] In total we included 371 patients with probable AD with 3T MRI FLAIR and T2* sequence present. We obtained informed consent from all patients to use their clinical data for research purposes.

Vascular risk factors

History of hypertension, hypercholesterolaemia, and diabetes mellitus were defined based on patient-reported medical history and medication use. In addition, systolic and diastolic blood pressure were measured manually using a sphygmomanometer, with the average of two measurements in supine position. For patients without supine measurements, blood pressure was measured once in sitting position. Smoking status was defined as never, former, or current smoking.

MRI protocol

MRI of the brain was acquired on a 3T whole body MR system (Signa, HDxt, General Electric Medical Systems, Milwaukee, WI, USA), using an 8-channel head coil. MRI protocol included an axial 2D T2* gradient-echo with an echo-planar read-out (EPI: matrix 256x480, field of view [FOV] 25x19cm^2, slice thickness 3.0mm, repetition time [TR] 5300ms, echo time [TE] 25ms, 2 excitations); a sagittal 3D fluid-attenuated inversion-recovery (FLAIR: matrix 224x224, FOV 25x25cm^2, slice thickness 1.2mm, TR 8000ms, TE 140ms); an axial 2D proton density/T2-weighted fast spin echo (PD-T2 matrix 384x384, FOV 25x19 cm^2, slice thickness 3.0mm, TR 9100ms, TE 23/114ms), and a 3D fast spoiled gradient recalled echo-based sequence (FSPGR; matrix 256x256, FOV 25x25 cm^2, slice thickness 1mm, TR 708ms) with oblique reconstructions.

MRI assessment

MRI rating was performed by an experienced neuroradiologist, blinded to clinical data. Microbleeds were counted on T2*-weighted sequences and were defined as small round foci of hypointense signal, up to 10mm in brain parenchyma. Lesions in sulci possibly

representing flow voids were excluded, as well as symmetrical lesions in the basal ganglia, suggestive for iron or calcium deposits. Hypointensities inside infarcts were regarded to be probable haemorrhagic transformations, and not counted as microbleeds. Microbleeds were counted in lobar (frontal, parietal, temporal and occipital) and non-lobar (basal ganglia including thalamus, and infratentorial) regions. Microbleed presence was dichotomized into absent (MB-: 0 microbleeds) or present (MB+: $\geq$1 microbleed). In addition, in microbleed-positive patients, patients with one microbleed were compared with patients with multiple microbleeds (>1). Finally we analysed microbleeds according to their location: strictly lobar, strictly non-lobar or mixed. On FLAIR sequences WMH were rated using the Fazekas scale[25] and classified as punctuate (grade 1); beginning confluent (grade 2) or confluent (grade 3). We dichotomized WMH score into minimal or absent WMH (WMH-: Fazekas<2; will be referred to as WMH absent) or WMH present (WMH+: Fazekas$\geq$2). Lacunar infarcts were defined as deep lesions (3-15mm), with (CSF-like) low signal on T1-weighted sequences and high signal on T2-weighted sequences; they were scored as absent or present. Medial temporal lobe atrophy (MTA) was rated on the oblique reconstructions of the FSPGR, using a 5-point rating scale (0-4).[26] Global cortical atrophy (GCA) was assessed on the FLAIR sequence, with a 4-point rating scale (0-3).[27] In the analyses, we dichotomized MTA scores into absent (mean left and right <1.5) or present (mean left and right $\geq$1.5). Similarly, we dichotomized GCA scores into absent (GCA<2) or present (GCA$\geq$2).

CSF analyses

Cerebrospinal fluid (CSF) was obtained by lumbar puncture between the L3/L4 or L4/L5 intravertebral space and was available for 239 (64%) patients. A 25 gauge needle was used and CSF was collected in 10mL polypropylene tubes (Sarstedt, Nümbrecht, Germany). Within two hours, CSF samples were centrifuged at 1800*g* for ten minutes at 4°C. A small amount of CSF was used for routine analyses, including total cells (leukocytes and erythrocytes), total protein, and glucose. CSF was aliquoted in polypropylene tubes of 0.5 and 1mL and stored at -20°C until further analysis. CSF Amyloid-Beta 1-42 (Aβ42) was determined with Innotest sandwich ELISA as described previously.[28] The performance of the assays was monitored with internal quality controls consisting of pools of surplus CSF specimens. During the study period multiple specimens with various concentrations have been used. The interassay coefficient of variation (CV) for CSF Aβ42 was (mean$\pm$SD) 11.3%$\pm$4.9%.

ApoE ε4 genotyping

DNA was isolated from 10mL of ethylenediaminetetra-acetic acid/blood and was available for 283 (76%) patients. Apolipoprotein E (ApoE) genotype was determined with the light cycler ApoE mutation detection method (Roche diagnostics GmbH, Mannheim, Germany). According to ApoE ε4 status patients were categorized into non-carriers, heterozygous, and homozygous carriers.

Statistical analyses

Statistical analyses were performed using SPSS 15.0 for windows (SPSS, Chicago, IL). Since levels of CSF Aβ42 were not normally distributed, we used the log-transformed value. Based on microbleed and WMH presence four groups were made (MB-WMH-; MB-WMH+; MB+WMH-; MB+WMH+). Group differences for patient characteristics, vascular risk factors, MRI variables, CSF Aβ42 levels, and ApoE ε4 status were investigated with one-way ANOVA with post-hoc Bonferroni and χ^2-test. Microbleed patients with and without WMH were directly compared using Student *t* test, χ^2-test, and Mann-Whitney *U* test. Associations of patient characteristics, vascular risk factors, MRI variables, and CSF Aβ42 (independent variables) with presence of microbleeds or WMH (dependent variables) were investigated with binary logistic regression. Similarly, these associations were analysed separately for strictly lobar microbleeds, strictly non-lobar microbleeds and microbleeds in mixed locations. For continuous variables, Odds ratios (ORs) were calculated per standard deviation increase. Multinomial logistic regression was used to assess associations for smoking status and ApoE ε4 dose. Regression models were adjusted for age and sex. We additionally adjusted for WMH in the models predicting presence of microbleeds and vice versa.

Results

In the total group of 371 AD patients, mean age was 69±9 years and 204 (55%) were female. Of all patients, 107 (29%) presented with WMH and microbleeds were present in 98 (26%). Of those, 67 (68%) had microbleeds in strictly lobar locations, 12 (12%) in strictly non-lobar locations and the remaining 19 (20%) had microbleeds in mixed locations. In total we counted 717 microbleeds, with 204 (28%) in frontal regions; 181 (25%) in parietal regions; 140 (20%) in temporal regions; 135 (19%) in occipital regions; 42 (6%) in infratentorial regions, and 15 (2%) in the basal ganglia, illustrating that in AD, the large majority of microbleeds have a lobar location.

Table 1. AD patients grouped according to microbleed and WMH presence

	MB-WMH- N=213	**MB-WMH+** N=60	**MB+WMH-** N=51	**MB+WMH+** N=47	**p-value**
Patient characteristics					
Age (yrs)	67±9	75±8*	68±9[#]	74±10*,[§]	<0.01
Sex (male)[a]	90(42%)	20(33%)	29(57%)*,[#]	28(60%)*,[#]	<0.05
Disease duration, yrs	3±2	3±2	3±2	3±2	n.s
MMSE	20.3±5.1	20.6±4.5	19.3±5.3	20.6±3.7	n.s.
Vascular risk factors					
Hypertension[a]	59(28%)	32(54%)*	17(33%)	21(47%)*	<0.01
Hypercholesterolaemia[a]	49(23%)	18(31%)	12(24%)	16(36%)	n.s.
Diabetes Mellitus[a]	19(9%)	7(12%)	4(8%)	7(16%)	n.s.
Smoking status[a]					n.s.
Never	119(58%)	29(52%)	27(55%)	22(51%)	
Former	54(27%)	15(27%)	15(31%)	15(35%)	
Current	30(15%)	12(21%)	7(14%)	6(14%)	
Systolic BP, mmHg	144.3±18.2	153.9±19.9*	152.8±22.4*	154.7±20.8*	<0.01
Diastolic BP, mmHg	82.5±9.4	83.7±12.7	86.5±11.1	85.1±11.5	n.s.
MRI					
GCA[a]	72(34%)	25(42%)	19(37%)	18(38%)	n.s.
MTA[a]	117(55%)	46(77%)*	31(61%)[#]	30(64%)	<0.05
Lacunes[a]	14(7%)	9(15%)*	2(4%)	13(28%)*,[§]	<0.01
CSF Aβ42, pg/ml	488±169	467±172	447±130	420±104	n.s.
ApoE ε4 status[a]					n.s.
Non-carriers	62(35%)	12(37%)	14(33%)	7(24%)	
Heterozygous	85(47%)	14(44%)	16(37%)	11(38%)	
Homozygous	32(18%)	6(19%)	13(30%)	11(38%)	

Abbreviations: MB-: 0 microbleeds; MB+: ≥1 microbleed; WMH-: Fazekas≤1; WMH+: Fazekas≥2; MMSE: Mini mental state examination; BP: blood pressure; GCA: global cortical atrophy; MTA: medial temporal lobe atrophy; CSF: cerebrospinal fluid.

Data are represented as mean±SD or number of patients with variable present (%). One-way ANOVA with post-hoc Bonferroni or χ^2 -test ([a]) were performed respectively.

Availability for incomplete data: *MB-WMH-*: MMSE: 210/213; Hypertension, Hypercholesterolaemia and Diabetes Mellitus: 210/213; Smoking: 203/213; BP: 178/213; CSF: 150/213; ApoE **ε**4: 179/213; *MB-WMH+*: Hypertension, Hypercholesterolaemia and Diabetes Mellitus: 59/60; Smoking: 45/60; BP: 34/60; CSF: 30/60; ApoE **ε**4: 32/60; *MB+WMH-*: MMSE: 50/51; Hypercholesterolaemia and Diabetes Mellitus: 50/51; Smoking:49/51; BP: 43/51; CSF: 33/51; ApoE **ε**4: 43/51; *MB+WMH+*: Hypertension, Hypercholesterolaemia and Diabetes Mellitus: 45/47; Smoking: 43/47; BP: 28/47; CSF: 26/47; ApoE **ε**4: 29/47.

*: p<0.05 compared to MB-WMH-; [#]: p<0.05 compared to MB-WMH+; [§]: p<0.05 compared to MB+WMH-.

Relative frequency of microbleeds in isolation (51 patients, 14%) and microbleeds in combination with WMH (47 patients, 13%) was comparable (see figure 1 for examples of both presentations). Demographics, clinical and MRI characteristics according to microbleed and WMH presence are given in table 1. Groups differed with regard to age ($p<0.01$), sex ($p<0.05$), history of hypertension ($p<0.01$), systolic blood pressure ($p<0.01$), MTA ($p<0.05$), and lacune presence ($p<0.01$). No group differences were found for the other variables.

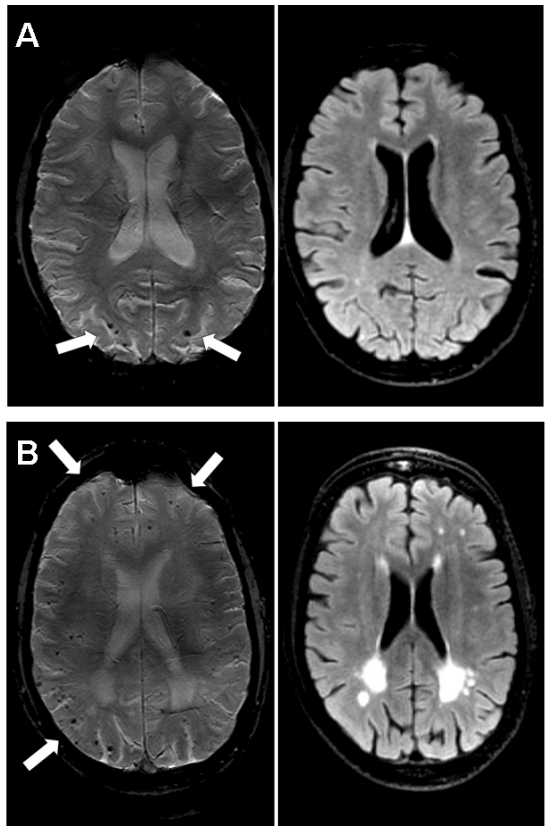

Figure 1.
Figure 1a: Example of a T2* and FLAIR MRI scan of a 46-year old female AD patient with microbleeds in the absence of white matter hyperintensities.
Figure 1b: Example of a T2* and FLAIR MRI scan of a 78-year old male AD patient with microbleeds accompanied by white matter hyperintensities.

In a direct comparison, patients with microbleeds and WMH in combination were older (74 ± 10 vs. 68 ± 9, $p<0.05$) and more frequently had lacunes (28% vs. 4%, $p<0.05$) than those with microbleeds in isolation. Moreover, patients with both microbleeds and WMH more often had multiple microbleeds ($p<0.05$) and tended to have a higher number of microbleeds ($p=0.08$) (table 2). Although the majority in both groups presented with strictly lobar microbleeds, patients with both microbleeds and WMH tended to have less strictly lobar microbleeds ($p=0.08$). All other variables, including vascular risk factors, CSF $A\beta42$ levels or ApoE $\varepsilon4$ status, did not differ between these two groups.

Table 2. Microbleeds in the absence or presence of white matter hyperintensities

	MB+WMH- N=51	**MB+WMH+** N=47	**p-value**
Median MBs	1(1-104)	3(1-98)	0.08
Multiple MBs [a]	24(47%)	32(68%)	<0.05
Strictly lobar MBs [a]	39(76%)	28(60%)	0.08
Strictly non-lobar MBs [a]	5(10%)	7(15%)	n.s.

Abbreviations: MBs: microbleeds; MB+: ≥1 microbleed; WMH-: Fazekas≤1; WMH+: Fazekas≥2.
Data are represented as median (range) or number of patients with variable present (%), with Mann-Whitney *U* test and χ^2-test respectively ([a]).

Subsequently, we assessed risk factors for the presence of microbleeds or WMH in age and sex adjusted models (table 3). Male gender (OR: 2.06; 95%CI 1.29; 3.30) and older age (OR: 1.27; 95%CI 1.00; 1.62) were associated with the presence of microbleeds. In addition, higher systolic (OR: 1.56; 95%CI 1.17; 2.08) and diastolic blood pressure (OR: 1.39; 95%CI 1.06; 1.83), and lower CSF Aβ42 (OR: 0.70; 95%CI 0.51; 0.96) were associated with microbleed presence. Finally, microbleeds were more prevalent in homozygous ApoE ε4 carriers compared to non-carriers (OR: 2.22; 95%CI 1.09; 4.55). When WMH was additionally adjusted for, the association between microbleeds and age disappeared (OR: 1.05; 95%CI 0.81; 1.36), but other associations remained essentially unchanged. Higher age (OR: 2.40; 95%CI 1.84; 3.13), history of hypertension (OR: 1.84; 95%CI 1.11; 3.00), current smoking (OR: 2.53; 95% CI 1.22; 5.27), higher systolic blood pressure (OR:1.48; 95%CI 1.09; 2.00), and presence of lacunes (OR: 2.74; 95%CI 1.31; 5.72) were associated with WMH. When microbleeds were added to the model, the association between systolic blood pressure and WMH became less strong (OR: 1.33; 95%CI 0.98; 1.81), whereas other associations remained comparable.

An additional analysis, in which risk factors for specific microbleed locations were analysed (table 4), showed that higher systolic (OR 1.62; 95% CI 1.16; 2.26) and diastolic blood pressure (OR 1.44; 95%CI 1.06; 1.98), and lower CSF Aβ42 (0.65; 95% CI 0.45; 0.95) were associated with strictly lobar microbleeds. Moreover strictly lobar microbleeds tended to be associated with ApoE ε4 homozygocity (OR 2.22; 95% CI 0.97; 5.08). When WMH were additionally adjusted for, the associations were slightly attenuated but remained comparable.

Strictly non-lobar microbleeds tended to be associated with current smoking (OR 3.88; 95% CI 0.99; 15.13), but after correction for WMH this tendency disappeared. Patients with microbleeds in mixed locations were more often homozygous ApoE ε4 carriers compared to non-carriers (OR 3.38; 95%CI 0.86; 13.24), although due to the small sample size, this effect did not reach significance.

Table 3. Associations with microbleeds and white matter hyperintensities

	Microbleeds (OR, 95% CI)	**WMH** (OR, 95% CI)
Patient characteristics		
Age (yrs)[a]	1.27 (1.00;1.62)	2.40 (1.84;3.13)
Sex (male)	2.06 (1.29;3.30)	0.99 (0.61;1.60)
MMSE[a]	0.82 (0.64;1.04)	1.98 (0.76;1.27)
Vascular risk factors		
Hypertension	1.19 (0.72;1,97)	1.84 (1.11;3.00)
Hypercholesterolaemia	1.05 (0.61;1.78)	1.28 (0.75;2.19)
Diabetes Mellitus	1.07 (0.50;2.30)	1.24 (0.59;2.63)
Smoking		
Former vs. never	1.17 (0.67;2.04)	1.55 (0.85;2.80)
Current vs. never	0.96 (0.46;1.98)	2.53 (1.22;5.27)
Systolic BP, mmHg[a]	1.56 (1.17;2.08)	1.48 (1.09;2.00)
Diastolic BP, mmHg[a]	1.39 (1.06;1.83)	1.20 (0.89;1.60)
MRI characteristics		
GCA	0.95 (0.57;1.56)	0.90 (0.54;1.51)
MTA	0.93 (0.56;1.54)	1.18 (0.70;2.01)
Lacunes	1.67 (0.81;3.46)	2.74 (1.31;5.72)
CSF Aβ42, pg/ml[a]	0.70 (0.51;0.96)	0.80 (0.59;1.09)
ApoE ε4 status		
Heterozygous vs. non-carrier	0.97 (0.50;1.86)	1.07 (0.54;2.13)
Homozygous vs. non-carrier	2.22 (1.09;4.55)	1.63 (0.75;3.54)

Abbreviations: WMH: white matter hyperintensities; MMSE: Mini mental state examination; BP: blood pressure; GCA: global cortical atrophy; MTA: medial temporal lobe atrophy; CSF: cerebrospinal fluid.

Data are represented as OR (95% CI), per standard deviation increase for continuous variables ([a]), or for the presence of the dichotomous variable. Models are adjusted for age and sex.

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Table 4. Associations with microbleed location

	Model 1			Model 2		
	Strictly lobar MBs	Strictly non-lobar MBs	Mixed MBs	Strictly lobar MBs	Strictly non-lobar MBs	Mixed MBs
Vascular risk factors						
Hypertension[a]	1.04 (0.58; 1.89)	1.33 (0.40; 4.50)	1.90 (0.70; 5.21)	0.93 (0.26; 3.28)	0.93 (0.26; 3.28)	1.57 (0.56; 4.37)
Hypercholesterolaemia[a]	1.11 (0.60; 2.05)	1.98 (0.59; 6.61)	0.50 (0.41; 1.82)	1.82 (0.53; 6.20)	1.82 (0.53; 6.20)	0.44 (0.12; 1.67)
Diabetes Mellitus[a]	1.04 (0.42; 2.57)	1.78 (0.36; 8.69)	1.03 (0.22; 4.85)	1.72 (0.34; 8.84)	1.72 (0.34; 8.84)	0.88 (0.18; 4.36)
Smoking status[a]						
Former vs. Never	1.28 (0.69; 2.40)	0.85 (0.15; 4.77)	0.99 (0.31; 3.12)	1.21 (0.64; 2.29)	0.74 (0.13; 4.20)	0.80 (0.24; 2.70)
Current vs. Never	0.72 (0.29; 1.79)	3.88 (0.99; 15.13)	0.39 (0.05; 3.26)	0.61 (0.24; 1.54)	2.79 (0.69; 11.27)	0.24 (0.03; 2.09)
Systolic BP, mmHg	1.62 (1.16; 2.26)	1.58 (0.77; 3.27)	1.68 (0.86; 3.30)	1.52 (1.08; 2.13)	1.34 (0.65; 2.78)	1.51 (0.75; 3.02)
Diastolic BP, mmHg	1.44 (1.06; 1.98)	1.59 (0.81; 3.10)	1.17 (0.63; 2.15)	1.39 (1.01; 1.90)	1.45 (0.74; 2.84)	1.10 (0.60; 1.99)
CSF Aβ42, pg/ml	0.65 (0.45; 0.95)	0.91 (0.42; 1.97)	0.69 (0.35; 1.33)	0.66 (0.44; 0.97)	0.98 (0.44; 2.16)	0.76 (0.39; 1.50)
ApoE ε4 status[a] (Non-carrier is ref.)						
Heterozygous	1.08 (0.51; 2.30)	0.99 (0.21; 4.61)	0.63 (0.13; 2.97)	1.05 (0.49; 2.25)	1.00 (0.21; 4.75)	0.68 (0.14; 3.28)
Homozygous	2.22 (0.97; 5.08)	1.24 (0.19; 7.85)	3.38 (0.86; 13.24)	2.13 (0.09; 4.93)	1.21 (0.19; 7.87)	3.23 (0.78; 13.39)

Abbreviations: MBs: microbleeds; BP: blood pressure; CSF: cerebrospinal fluid.

Data are represented as OR (95% CI), per standard deviation increase for continuous variables ([a]), or for the presence of the dichotomous variable. Model 1: adjusted for age and sex; Model 2: adjusted for age, sex and white matter hyperintensities.

Availability for incomplete data: *Strictly lobar microbleeds*: Hypertension, Hypercholesterolaemia and Diabetes Mellitus: 66/67; Smoking: 40/67; BP: 51/67; CSF: 41/67; ApoE **ε**4: 50/67; *Strictly non-lobar microbleeds:* Smoking: 10/12; BP: 9/12; CSF: 7/12; ApoE **ε**4: 9/12; *Mixed microbleeds*: Hypertension: 18/19; Hypercholesterolaemia and Diabetes Mellitus: 17/19; Smoking: 12/19; BP: 11/19; CSF: 11/19; ApoE **ε**4: 13/19.

Discussion

In this sample of relatively young AD patients, we showed that almost half presented with microbleeds and/or WMH. Microbleeds in isolation were equally common as microbleeds accompanied by WMH. WMH were independently associated with higher age, hypertension, smoking, and lacunes, whereas microbleeds were independently related to male gender, higher blood pressure, lower CSF Aβ42, and ApoE ε4 homozygocity. The associations for microbleeds were mostly attributable to microbleeds in lobar locations. We found no differences in risk factor profile for patients that presented with microbleeds in isolation or with additional WMH.

In the present study we observed that microbleeds in AD are independently related to lower CSF Aβ42 and ApoE ε4 homozygocity. These findings corroborate our previous reports[18,29] and support the notion that microbleeds in AD reflect increased amyloid burden. In addition to amyloid deposition in the brain parenchyma as plaques, amyloid may deposit in cerebral blood vessel walls as CAA.[30] As both groups of patients clinically suffer from AD with comparable disease duration and severity, we speculate that the additional amyloid burden may reflect CAA. WMH showed associations with ageing, lacunes, and vascular risk factors. Previous studies have indicated that histopathological correlates for WMH in AD are heterogeneous, ranging from axonal loss and demyelination to gliosis.[13,31-33] Similar tissue changes have also been found in the white matter of CAA patients.[34] In AD patients, however, only some studies find weak correlations between WMH and CAA,[35] whereas others fail to find associations at all.[33,36] WMH in AD may therefore be more related to underlying small vessel disease than CAA.

The majority of our AD patients presented with microbleeds in strictly lobar regions. This confirms previous reports from our group and others[29,37] and seems in line with the hypothesis that CAA explains the frequent occurrence of microbleeds in AD. In the additional analysis investigating microbleed location, risk profiles for microbleeds in mixed locations appeared rather similar to risk profiles for strictly lobar microbleeds, although it should be taken into account that risk estimates did not reach significance due to low power. One might argue that in AD, the presence of *any* lobar microbleed, rather than *strictly* lobar microbleeds, reflects CAA. Strictly non-lobar microbleeds tended to be more closely associated with vascular risk factors, although no significance was reached due to low power. Microbleeds in lobar locations, however, related to blood pressure as well. A recent study investigating microbleeds in different locations with Positron Emission Tomography (PET), with Pittsburgh compound B (PiB), also showed that microbleeds in lobar locations related to both amyloid deposition and small vessel disease burden.[38] Moreover, CAA may co-occur with hypertension.[20,39] Possibly, the

presence of mixed pathology in AD hampers the clear disentangling of different risk factors in microbleeds and relate them to specific locations.

A direct comparison of microbleed patients with and without additional WMH showed that patients with both microbleeds and WMH were older and presented more frequently with lacunes and multiple microbleeds. Prevalence of vascular risk factors as well as CSF Aβ42 levels and ApoE ε4 status were, however, comparable. Moreover, contrary to previous findings,[11,12,18,29] we did not find an evident difference in location for microbleeds with or without additional WMH. As vascular pathology has been related to ageing,[40] the presence of both microbleeds and WMH, as well as a higher prevalence of lacunes in this group, may primarily result from the patients being older.

At present it is recommended that AD patients with more than four microbleeds are excluded from anti-amyloid immunization trials.[22] Microbleeds are seen as a side effect of these trials, but evidence about the risk that baseline microbleeds convey is still insufficient.[41] It is hypothesized that immunization results in removing Aβ42 plaques from the brain via the perivascular pathway, resulting in a transient increase in vascular Amyloid-Beta 1-40 (Aβ40) and CAA.[21] Moreover, retrospective analyses of trials indicated that ApoE ε4 carriership predisposes to developing ARIA. Homozygous ApoE ε4 carriers in particular, had a seven-fold increased risk of developing ARIA compared to non-carriers.[41] Associations with lower levels of CSF Aβ42 and ApoE ε4 homozygocity indicate that the presence of lobar microbleeds in AD reflects additional amyloid burden. AD patients with lobar microbleeds are, therefore, presumably more at risk for developing ARIA than AD patients without.

A strength of the present study consists of the large cohort of AD patients with available 3T MRI FLAIR and T2* scans. As neuropathological confirmation of the diagnosis of AD was only available for one patient, the possibility that some patients in fact did not suffer from AD cannot be excluded. However, all subjects fulfilled criteria for AD, and the majority were positive for CSF biomarkers of AD pathology; namely, reduced levels of Aβ42 combined with increased levels of tau and p-tau. As radiologically defined microbleeds frequently lack histopathological evaluation, microbleed research often depends on associations with surrogate markers to infer aetiology. As previously shown, a relation between microbleeds and amyloid pathology could be more thoroughly investigated in vivo with the use of PiB-PET imaging.[38,42]

Our results suggest that microbleeds in AD patients are associated with risk factors reflecting additional amyloid burden, and as such, may reflect CAA. WMH in AD, on the other hand, seem less specific for amyloid burden and relate more to conventional vascular risk factors. Moreover, it would be conceivable that in AD, not just the presence of *strictly* lobar microbleeds, but rather *any* lobar microbleed, may reflect CAA.

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